(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) EP 1 000 541 A1

(12) EUROPEAN PATENT APPLICATION

(43) Date of publication:
17.05.2000 Bulletin 2000/20

(51) Int Cl.7: **A01N 1/02**, C12N 5/00

(21) Application number: **99308702.2**

(22) Date of filing: **02.11.1999**

(84) Designated Contracting States:
AT BE CH CY DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE
Designated Extension States:
AL LT LV MK RO SI

(30) Priority: **05.11.1998 US 186580**

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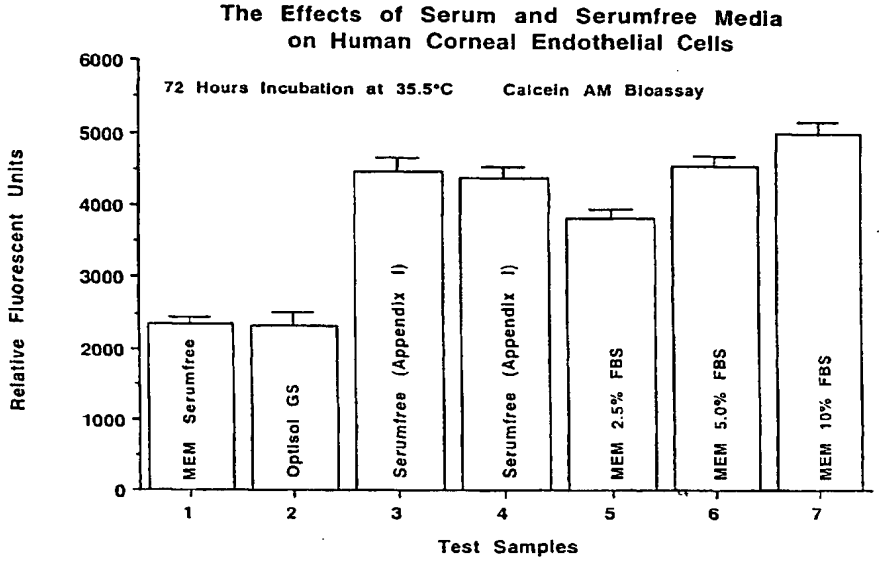
(54) Defined serumfree medical solution for ophthalmology

(57) A defined serumfree medical solution for applications in Ophthalmology, that contains one or more cell nutrient supplements, and a growth factor(s) which maintains and enhances the preservation of eye tissues, including human corneal, retinal and corneal epithelial tissues at low to physiological temperatures (2°C to 38°C). This solution is composed of a defined aqueous nutrient and electrolyte solution, supplemented with a glycosaminoglycan(s),a deturgescent agent(s), an energy source(s), a buffer system(s),an antioxidant(s), membrane stabilizing agents, an antibiotic(s) and/or antimycotic agent(s), ATP or energy precursors, nutrient cell supplements, coenzymes and enzyme supplements, nucleotide precursors, hormonal supplements, non-essential amino acids, trace minerals, trace elements and a growth factor(s).

FIGURE 1



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Description

CROSS REFERENCES TO CO-PENDING APPLICATIONS

[0001] None

BACKGROUND OF THE INVENTION

[0002] 1. **Field of the Invention** - The present invention relates to the preservation of eye tissue in a defined serumfree medical solution, and more particularly, relates to the preservation and enhancement of human corneal tissue, specified as the time between removal from the donor and transplantation.

[0003] 2. **Description of the Prior Art** - Keratoplasty, or the transplantation of the cornea, has been effective in providing visual rehabilitation to many who suffer from corneal disorders. This procedure has been severely hampered by the universally inconsistent availability of donor tissue. The use of 4°C corneal storage medium containing chondroitin sulfate has positively impacted the availability of quality donor tissue. In the United States 95% of all corneas transplanted are stored in a 4°C chondroitin sulfate containing medium for up to seven days. After 96 hours of preservation the cornea is attended by epithelial decomposition and loss of corneal clarity, as demonstrated by increased swelling of the corneal stroma. The stromal edema is attributed to both the decreased maintenance of the barrier pump function of the corneal endothelium and barrier function of the corneal epithelium.

[0004] An alternative to 4°C corneal storage is the use of organ culture. In this method of corneal preservation, the cornea is maintained at higher temperatures (31°C-37°C) allowing greater metabolic activity of the cornea. The use of organ cultured corneas is mainly supported in Europe. The organ culture system utilizes fetal bovine as a major medium component. Mounting concerns over TSEs (Transmissble Spongiform Encephalopathies) stemming from Bovine Spongiform Encephalophy (BSE) outbreaks, have focused much emphasis on animal derived products and their use in corneal preservation. The replacement of serum components in corneal preservation is a formidable challenge, based on over 350 known chemical components found in serum.

[0005] The elevated temperature (31°C-37°C) of the organ culture technique increases the metabolic rate of the cornea as compared to corneas stored at 4°C. The corneal storage medium must provide an environment similar to the in vivo situation. A serumfree corneal preservation medium must be completely defined as to supplement the components normally found in serum. A critical evaluation of physiologic parameters such as ionic and amino acid composition, bicarbonate equilibrium, available energy sources, dissolved oxygen levels, nutrient cell supplements, coenzymes and enzyme supplements, nucleotide precursors, hormonal supplements, trace minerals, trace elements, growth factors, osmolality and pH should be observed with respect to each preservation medium. Parameters for extended serumfree organ culture preservation should be defined as to the reversibility of cell damage incurred during storage.

[0006] Adult corneal endothelium have a limited regenerative capacity and mitotic figures have been rarely observed in vivo; human corneal endothelium in vivo normally responds to trauma by sliding into the wounded area by cell migration. However, in vivo endothelial cell mitosis has been demonstrated in rabbit, bovine and human endothelium. Autoradiographic thymidine uptake studies after cryowounding or mechanical wounding of corneas in vitro has demonstrated existence of mitotic figures in the endothelial monolayer. These studies were all conducted in the presence of serum. Surgical trauma and disease can accelerate the loss of endothelial cells and further compromise the cornea. Thus, the long term preservation and enhancement of the corneal endothelium is a very important aspect of eye bank storage of eye tissue.

[0007] An overview of the issues surrounding the storage and handling of corneal tissue is found in Corneal Surgery, chapters 1-4, pages 1-128 edited by Federick S. Brightbill, M.D., published by C.V. Mosby Company, St. Louis, MO, 1986. A variety of storage media and techniques have been proposed, and current research continues to be directed towards maintaining and actually enhancing the quality of the donor tissues, and increasing the duration of storage of corneal tissues, as defined as the time between excision from a donor and transplantation. Currently, there are no defined serumfree media used in organ culture techniques at 31°C-38°C.

[0008] Accordingly, the present inventions directed towards materials and methods of enhancing ocular tissues, especially corneal tissues, during storage prior to transplantation. One aspect of the invention provides for the enhancement of corneal tissue viability by providing a completely defined serumfree medium that maintains normal physiologic metabolism, and maintains corneal tissue equal to medium that contains serum.

SUMMARY OF THE INVENTION

[0009] Organ culture corneal storage at 31°C-37°C should provide tissue preservation which is capable of sustaining the functional status of the endothelium. Experimental work has demonstrated that the defined serumfree medical

solution is capable of maintaining corneas equal to that of solutions containing serum. The undesirable attributes of storage in serum containing solutions are avoided. The present invention has defined those components that are necessary to maintain corneal tissues during organ culture. The present invention further defines a nutritive solution that provides the corneas with a glycosaminoglycan(s), a deturgescent agent(s), an energy source(s), a buffer system (s),an antioxidant(s), membrane stabilizing agents, an antibiotic(s) and/or antimycotic agent(s), ATP or energy precursors, nutrient cell supplements, coenzymes and enzyme supplements, nucleotide precursors, hormonal supplements, non-essential amino acids, trace minerals, trace elements and a growth factor(s) that enhance cell metabolism, wound healing and cell viability. Cell proliferation is regulated by events leading to DNA synthesis; whether or not a cell proceeds with DNA synthesis or is arrested in the early stages of the cell cycle is dependent upon extracellular conditions. Cellular metabolism can be enhances by the addition of essential nutritive components by increasing hexose transport, protein synthesis, amino acid and ion transport.

[0010] The novel defined nutrient containing solutions are serumfree. These solutions are able to be used as human corneal preservation solutions, that maintain human corneas equal to solutions containing serum. While serum-supplemented solutions can stimulate mitosis in human corneal cells in tissue culture, the presence of serum in products for use with tissues for human transplantation presents many disadvantages. Serum can be an agent for the transmission of many diseases, such as viral diseases, most notably TSEs (Transmissible Spongiform Encephalopathies). Non-human-derived serum contains many substances capable of eliciting an immune response, and all sera contain some substances such as endotoxins, and growth factors that actually retard cell mitosis. Corneal preservation solutions are well known. Commercially available serumfree corneal storage media for 4°C preservation consist of Optisol and Optisol-GS are available from Bausch and Lomb, Surgical (Irvine, CA). These medium were developed by D.L. Skelnik, B.S., and R.L. Lindstrom, M.D. Commercially available serum containing medium for organ culture are available from Opsia (France). No serumfree media for organ culture are available or in current use.

[0011] Nutrient and electrolyte solutions are well defined in the art of tissue culturing. Such solutions contain the essential nutrients and electrolytes at minimal concentrations necessary for cell maintenance and cell growth. The actual compositions of the solutions may vary greatly. In general, they contain inorganic salts, such as calcium magnesium, iron, sodium and potassium salts of carbonates, nitrates, phosphates, chloride, and the like, essential and non-essential amino acids and other essential nutrients. Chemically defined basal nutrient media are available, for example, from Gibco BRL (Grand Island, NY) and Sigma (St. Louis, MO) under the names Minimal Essential Medium and TC199. Corneal storage solutions have been adapted from these nutrient media. The defined serumfree medical solution base of the present invention is composed of components found in both MEM and TC199 supplemented with glycosaminoglycan(s), a deturgescent agent(s), an energy source(s), a buffer system(s),an antioxidant(s), membrane stabilizing agents, an antibiotic(s) and/or antimycotic agent(s), ATP or energy precursors, nutrient cell supplements, coenzymes and enzyme supplements, nucleotide precursors, hormonal supplements, non-essential amino acids, trace minerals, trace elements and a growth factor(s).

BRIEF DESCRIPTION OF THE DRAWINGS

[0012] Other objects of the present invention and many of the attendant advantages of the present invention will be readily appreciated as the same becomes better understood by reference to the following detailed description when considered in connection with the accompanying drawings, in which like reference numerals designate like parts throughout the figures thereof and wherein:

[0013] **FIG. 1** - The Effects of Serum and Serumfree Media on Human Corneal Endothelial Cells.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

[0014] **FIG. 1** - Preferred defined serumfree medical solutions for use in the composition and methods of this invention contain an aqueous nutrient and electrolyte solution (e.g. Minimal Essential Medium and/or TC199 medium; a glycosaminoglycan (e.g. chondroitin sulfate, dermatin sulfate, heparin sulfate, heparan sulfate, keratan sulfate and/or hyaluronic acid) in the range of .001 mg/ml to 1.0 gram/ml; a deturgescent agent (e.g. dextran, dextran sulfate, hydroxypropylmethyl cellulose, carboxymethylcellulose, cell gum, sodium alginate, albumin, hydroxyethyl starch, hydroxethyl cellulose, dextrose, glucose and/or cyclodextrin) in the range of .001 mg/ml to 1.0 gram/ml; an energy source (glucose, pyruvate, sucrose, fructose and/or dextrose) in a range of .01 mM to 10 mM; a buffer system (e.g. sodium bicarbonate, sodium acetate, sodium citrate, sodium phosphate and/or HEPES buffer) in a range of .01 mM to 10 mM; an antioxidant (e.g. L-ascorbic acid, 2-mercaptoethanol, glutathione, alpha-tocopherol, alpha-tocopherol acetate, alpha-tocopherol phosphate, and/or selenium)in a range of .001 μM to 10 mM; a membrane stabilizing component (e.g. vitamin A, vitamin B, retinoic acid, trans-retinoic acid, retinol acetate, ethanolamine, phosphoethanolamine, transferrin, lecithin, B-sitosterol and/or L-α-phosphatidyl choline) in a range of .001 pg/ml to 500 mg/ml; h. an antibiotic an/or antimycotic (e.g. gentamycin, kanamycin, neomycin, vancomycin, tobramycin, dindamycin, streptomycin, levo-

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floxacin, penicillin, cyclosporin, amphotericin B and/or nystatin) in the range of .001 µg/ml to 100 mg/ml; ATP or energy precursors (e.g. adenosine, inosine, adenine, flavin adenine dinucleotide, uridine 5'-triphosphate Na, 5' methylcytosine, *B*-NAD and/or *B*-NADP Na) in the range of .001 mM to 10 mM; nutrient cell supplements (e.g. alynyl-glutamine, glycyl-glutamine, L-amino-n-butyric acid, L-arginine, D-biotin, betaine HCl, D-carnitine, calciferol, carotene, cholesterol, L-cystine, L-cystiene, L-glutamic acid, D-glucosamine, glucuronate, D-glucuronolactone, L-hydroxyproline, hypoxanthine, L-inositol, glycine, L-ornithine, L-proline, L-serine, myo-inositol, menadione, niacin, nicotinic Acid, p-amino benzoic acid, D-panthothenic Acid, pyridoxal-5-phosphate, pyridoxine HCl, taurine, thymidine, xanthine and or vitamin B12) in a range of .001 µM to 10 mM; coenzymes and enzyme supplements (e.g. acetyl coenzyme A, cocarboxylase, coenzyme A, coenzyme Q10 and/or coenzyme K) in a range of .001 µM to 10 mM; nucleotide precursors (e.g. 2' deoxyadenosine, 2' deoxycytidine HCL, 2' deoxyguanosine, 2-deoxy-D-ribose and/or D-ribose) in a range of .001 µM to 10 mM; hormonal supplements (e.g. *B*-estradiol, progesterone, testosterone, cortisol, corticosterone, thyroxine, thyroid stimulating hormone and/or calcitonin) in a range of .001 pg/ml to .100 mg/ml; non-essential amino acids (e.g. L-alanine, L-asparagine, L-aspartic acid, L-glutamic acid, glycine, L- proline and/or L-serine) in the range of .001 µg/ml to 100 mg/ml; trace minerals and trace elements (e.g. $CuSO_4$ $5H_20$, $ZnSO_4$ $7H_2O$, Selenite Na, Ferric citrate, $MnSO_4$ H_20, $NaSIO_3$ $9H_20$, molybdic acid, NH_4VO_3, $NiSO_4$ $6H_2O$, $SnCl_2$, $AgNO_3$, $Ba(C_2H_3O2)_2$, KBr, $CdCl_2$, $CoCl_2$, $CrCl_3$, NaF, GeO_2, KL, RbCl, $ZrOCl_28H_20$) in the range of .001 pg/ml to .100 mg/ml; p. Growth factors (animal, animal recombinant, human recombinant or natural);(PDGF-BB, PDGF-AA, Nerve growth factor, Nerve growth factor, Stem cell factor, Transforming growth factor-α, Transforming growth factor-B, Vascular endothelial growth factor, B-endothelial cell growth factor, Epidermal growth factor, Epithelial neutrophil activating peptide, Heparin binding EGF-like growth factor, Fibroblastic growth factor-acidic, Fibroblastic growth factor-basic, IGF-I, IGF-II, Keratinocyte growth factor, Platelet-derived endothelial cell growth factor, Insulin) in the range of .001 pg/ml to .100 mg/ml.

[0015] The serumfree medical solution of this invention is composed of a defined aqueous nutrient and electrolyte solution, supplemented with a glycosaminoglycan(s),a deturgescent agent(s), an energy source(s), a buffer system (s),an antioxidant(s), membrane stabilizing agents, an antibiotic(s) and/or antimycotic agent(s), ATP or energy precursors, nutrient cell supplements, coenzymes and enzyme supplements, nucleotide precursors, hormonal supplements, non-essential amino acids, trace minerals, trace elements and a growth factor(s) in the amounts sufficient to enhance cell metabolism, cell viability and wound healing following organ culture storage. The excised corneas are aseptically transferred to containers of the corneal storage solution, which are then sealed. For storage these corneas are stored at (2°C to 38°C) optimally at 31°C-37°C). These corneas are stored for up to 28 days, changing the medium at day 14. At the time of transplantation the corneas are thinned down with solution containing a deturgescent agent. At the time of transplantation, normal corneal deturgescence is maintained intraoperatively and post-operatively. Endothelial function and metabolism is maintained, permitting permanent hydration of the cornea, and thus constant thickness and transparency post operatively. In addition to providing a viable cornea for transplantation, wound healing is potentiated. Various modifications can be made to the present invention without departing from the apparent scope thereof. For instance, the serumfree medical solution can be used in any medical application, and is not strictly limited to ophthalmology. The invention is further illustrated by the following examples, which is not intended to be limiting. not strictly limited to ophthalmology. The invention is further illustrated by the following examples, which is not intended to be limiting.

MODE OF OPERATION

[0016] Organ culture preservation should provide tissue preservation capable of sustaining the functional status of the corneal endothelium. Each of the components listed in Appendix I were tested in cell culture models with human corneal endothelium, human corneal stromal keratocytes and human corneal epithelial cells to determine optimal concentrations. The following examples are based on the final formulation to illustrate the effect that the formulation had on these cell types. Once the optimum concentrations were derived in cell culture models, test formulations were then tested on human corneas.

Example One

The Effects of A Defined Serumfree Medical Solution and Serum Containing Medium On Human Corneal Endothelial Cells

[0017] Standard organ culture medium utilizes MEM supplemented with 2.0% fetal bovine serum. A serumfree medium that is to be used for organ culture must support human corneal endothelial cell growth equal to MEM supplemented with 2.0% FBS. This study was conducted to evaluate the defined serumfree medical solution (Appendix I) for human corneal endothelial cell growth against serumfree MEM, MEM containing 2.5% FBS, 5.0% FBS, 10% FBS and commercial Optisol-GS. The test solutions were evaluated in a fluorogenic Calcein AM bioassay with human corneal

endothelial cells (HCE). Isolation techniques developed in our laboratory have enabled the establishment of primary and subsequent subcultures of human corneal endothelial cells. In *vitro* conditions maintain these human corneal endothelial cells in a proliferative state, actively undergoing mitosis. Cell culture offers a model system in which these cells can be studied. A quantitative bioassay has been developed to determine the effects of various test solutions on the stimulation or inhibition of cell division of endothelial cells as measured by Calcein esterase quantitation. A fluorogenic Calcein AM bioassay was used to measure total esterase enzyme activity that is directly proportional to cell number. A Wilcoxen Signed-Rank Test was used to evaluate statistical significance ($p<0.05$) between the test and control groups. This study was performed at Insight Biomed, Inc., Minneapolis, MN.

Calcein AM Fluorescent Quantitative Bioassay

[0018] Live cells are distinguished by the presence of ubiquitous intracellular esterase activity, determined by the enzymatic conversion of the virtually non-fluorescent cell permeant calcein AM to the intensely fluorescent calcein. The polyanionic calcein is well retained within live cells, producing an intense uniform green (530 nm) fluorescence in live cells.

Calcein AM (Non-Fluorescent) + Esterases = Calcein (Fluorescent Product)

[0019] Calcein, which is the esterase product of Calcein AM, is a polar fluorescein derivative that is better retained by viable cells and is 2.5 times brighter than BCECF. The excitation and emission maxima are 485 nm and 530 nm respectively.

Human Corneal Endothelial Cell Cultures

[0020] Ninety-six well tissue culture plates were seeded with 1 $X10^3$ cells/well in a final volume of 200 µl of designated medium. Third passage HCE cells were maintained in a humidified incubator at 35.5°C in a 95% air: 5% CO_2 atmosphere. After 1 day of incubation in CSM, supplemented with 10% fetal bovine serum, the medium was removed. The cells were then rinsed one time and incubated with the appropriate test or control solutions. HCE cells were incubated for 72 hours. At the end of each time interval, each well was then rinsed two times with 200 µl of Dulbecco's modified phosphate buffered saline. HCE cells were then incubated with 100 µl/well of 2 µM Calcein AM solution (Molecular Probes, Inc. Eugene, OR) and immediately read on a Millipore CytoFluor™ 2300 Fluorescence Measurement System. A 485/20 nm excitation wavelength and the 530/25 nm emission wavelength filter set (sensitivity 5) was used to measure the fluorescent product. A Wilcoxen Signed-Rank Test was used to evaluate statistical significance ($p<0.05$) between the test and control groups.

Results				
Statistical Significance as compared to 2.5%($p<0.05$)				
—	RFU			
MEM	**2356 ± 96**	Yes	.0022	less than
OPTISOL-GS	**2339 ± 184**	Yes	.0022	less than
Serumfree (Appendix I)	**4460 ± 205**	Yes	.0022	greater than
Serumfree (Appendix I)	**4474 ± 168**	Yes	.0022	greater than
MEM 2.5% FBS	**3832 ± 122**			
MEM 5.0% FBS	**4554 ± 141**	Yes	.0022	greater than
MEM 10% FBS	**5031 ± 163**	Yes	.0022	greater than

Discussion

[0021] This study was conducted to evaluate the defined serumfree medical solution (Appendix I) for human corneal endothelial cell growth against serumfree MEM, MEM containing 2.5% FBS, 5.0% FBS, 10% FBS and commercial Optisol-GS. The test solutions were evaluated in a fluorogenic Calcein AM bioassay with human corneal endothelial cells (HCE). A quantitative bioassay has been developed to determine the effects of various test solutions on the stimulation or inhibition of cell division of endothelial cells as measured by Calcein esterase quantitation. A fluorogenic Calcein AM bioassay was used to measure total esterase enzyme activity that is directly proportional to cell number. A Wilcoxen Signed-Rank Test was used to evaluate statistical significance ($p<0.05$) between the test and control

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groups.

[0022] Human corneal endothelial cells incubated with solutions MEM and Optisol GS exhibited a statistically significant decrease in total Calcein fluorescence as compared to the MEM 2.5% FBS control medium. Human corneal endothelial cells incubated with defined serumfree medical solutions (Appendix I) exhibited a statistically significant increase in total Calcein fluorescence as compared to the MEM 2.5% FBS control medium. Human corneal endothelial cells incubated with MEM 5.0% FBS and MEM 10.0% FBS exhibited a statistically significant increase in total Calcein fluorescence as compared to the MEM 2.5% FBS control medium. In conclusion from this data the defined serumfree medical solution (Appendix I) was capable of maintaining total Calcein fluorescence (total number of HCE cells) statistically greater than MEM 2.5% FBS control medium. Therefore, this solution is acceptable for use in organ culture as a corneal preservation solution for human corneal transplantation as defined by the parameters of this bioassay.

Example Two

A Comparative Study of A Serumfree Medical Solution and Standard MEM 2% FBS Medium With Human Corneas

[0023] Human donor corneas were immersed in 1% povidone iodine in normal saline for three minutes, followed by a one-minute immersion in normal saline. The globes were then rinsed with 12 cc of normal saline with a syringe fitted with a 18-gauge needle. Twenty paired corneas from donors unsuitable for transplantation because of age or cause of death were removed at a certified eye bank an average of 12.0 hours after death and placed in commercial Optisol-GS (Bausch and Lomb, Surgical) at 4°C. Donor globes were transported to the research lab. One of each pair was placed into 100 ml of serumfree (Appendix I) medium. The paired cornea was placed in 50 ml of MEM, containing L-glutamine, HEPES, penicillin , streptomycin, amphotercin B and 2% FBS. Corneas were suspended on a 4.0 silk suture. Each of the bottles containing the corneas were closed and kept at 35°C for 14 days. At this time, 10 pairs of corneas were removed and placed in appropriate fresh medium and stored for another 14 days. At the 14 day and 28 time points, corneas stored in serumfree (Appendix I) medium were then placed in commercial Optisol-GS at 35°C for 24 hours. The paired cornea stored in MEM 2% FBS were placed in MEM containing 6% T500 dextran at 35°C for 24 hours. Due to the increased hydration of the cornea at elevated temperatures, corneas needed to be thinned down by this procedure. Corneas were evaluated after thinning by the following methods. The corneal thickness was measured by microscopic evaluation with a micrometer. The corneal endothelium was evaluated by staining with 0.1% trypan blue and alizarin red S after the final corneal thickness measurements were taken. Corneal thickness at the 14 day incubation period were .386 ± .049 mm and .479 ± 078 mm, respectively, for the serumfree (Appendix I) medium and MEM 2%. Corneas stored in the serumfree (Appendix I) medium demonstrated a statistically significant ($p<.05$) decrease in corneal thickness over corneas stored in the MEM 2% FBS medium. Corneas stored in the serumfree (Appendix I) medium had endothelial cell counts of 2716 ± 712 cells/mm^2 as compared to 2573 ± 753 cells/mm^2 for corneas stored in MEM 2% FBS. There was no statistical difference between these two groups with relation to endothelial cell counts. All endothelial cell monolayers were intact, with normal endothelial cell morphology for both the serumfree (Appendix I) medium and the MEM 2% FBS stored groups. Corneal epithelium was intact for both groups. Corneal thickness at the 28 day incubation period were .343 ± .015 mm and .379 ± 015 mm, respectively, for the serumfree (Appendix I) medium and MEM 2%. Corneas stored in the serumfree (Appendix I) medium demonstrated a statistically significant ($p<.05$) decrease in corneal thickness over corneas stored in the MEM 2% FBS medium. Corneas stored in the serumfree (Appendix I) medium had endothelial cell counts of 2451 ± 617 cells/mm^2 as compared to 2422 ± 570 cells/mm^2 for corneas stored in MEM 2% FBS. There was no statistical difference between these two groups with relation to endothelial cell counts. All endothelial cell monolayers were intact, with normal endothelial cell morphology for both the serumfree (Appendix I) medium and the MEM 2% FBS stored groups. Corneal epithelium was intact for both groups.

[0024] In conclusion, from the results of this comparative study, corneas stored for both 14 and 28 days in serumfree (Appendix I) medium were able to maintain viable corneal endothelium equal in performance to corneas stored in MEM 2% FBS. This serumfree (Appendix I) medium was effective in maintaining normal corneal cell function and metabolism. Therefore, this serumfree (Appendix I) medium is therefore, acceptable for use as an organ culture preservation medium.

[0025] Various modifications can be made to the present invention without departing from the apparent scope hereof.

Appendix 1, Defined Serumfree Medical Solution, is attached.

APPENDIX I

Components	grams/liter
Calcium Chloride 2H20	.106000
Calcium Chloride Anhydrous	.105511
Magnesium Sulfate (Anhydrous)	.091526
Potassium Chloride	.371023
Sodium Acetate (anhydrous)	.012000
Sodium Chloride	6.069984
Sodium Phosphate Monobasic (anhydrous)	.122658
Ferric Nitrate	.000301
L-Alanine	.017932
L-Arginine HCL	.078936
L-Asparagine H20	.012676
L-Aspartic Acid	.011944
L-Cystine 2 HCL	.021979
L-Glutamic Acid	.012124
L-Glutamine	.054292
Glycine	.009904
L-Histidine HCl H20	.032817
Hydroxy-L-Proline	.007629
L-Isoleucine	.034649
L-Leucine	.035609
L-Lysine HCL	.053620
L-Methionine	.009689
L-Ornithine HCL	.003764
L-Phenylalanine	.023494
L-Proline	.009352
L-Serine	.010600
L-Threonine	.032895
L-Tryptophan	.012276
L-Tyrosine 2NA 2H20	.036860
L-Valine	.034268
Adenine sulfate	.005993
Adenosine	.003007
L-Ascorbic Acid Na	.020030
D-Biotin	.000016
Calciferol	.000158
Choline Chloride	.001028
Folic Acid	.000538
i-Inositol	.001055
Inosine	.005993
Myo-Inositol	.000050
Menadione (Sodium Bisulfite)	.000016
Niacin	.000015
Niacinamide	.000553
Nicotinic Acid	.000025
P-Amino Benzoic Acid	.000060
D-Ca Pantothenate	.000528
D-Panthothenic Acid (Hemicalcium)	.000010
Pyridoxal HCL	.000553
Pyridoxine HCL	.000175
Retinol Acetate	.000100
Riboflavin	.000063
Thiamine HCL	.000538
DL-α-Tocopherol Phosphate 2 Na	.000016
Vitamin B-12	.004618
L-Amino-n-Butyric Acid	.002204
Cocarboxylase	.000400
Coenzyme A Na	.001000
2'-Deoxyadenosine	.004000
2'-Deoxycytidine HCL	.004000
2'-Deoxyguanosine	.004000
Flavin Adenine Dinucleotide 2 NA	.000400
D-Glucosamine HCL	.001540
D-Glucose	.927557
Glucuronate Na	.000720
D- Glucuronolactone	.000720
Glutathione Na	.000000
5' Methylcytosine HCl	.000040
B-NAD	.002800
B-NADP Na	.000400
Phenol Red Na	.013276
Taurine	.001672
Thymidine	.004000
Tween 80	.005000
Uridine 5'-Triphosphate Na	.000400
HEPES	3.143182
Cholesterol	.000120
Sodium Bicarbonate	2.320000

Components	grams/liter
Sodium Pyruvate	.0660000
Gentamycin	.0900000
Streptomycin	.1200000
2-Mercaptoethanol	.3mM
Chondroitin Sulfate	3.000000
L-Ascorbic Acid	.010566
L-Alynyl-L-Glutamine	2 mM
Glutathione Na Reduced	.307000
(+)-α-Tocopherol Acetate	.441120
Recombinant human Insulin	.006000
Recombinant human PDGF-BB	.000200
B-Estradiol	.000001
Progesterone	.000002
D-Carnitine HCl	.002500
Pyridoxal-5-Phosphate	.001000
Betaine HCL	.001250
L-α-Phosphatidyl Choline	.000500
Hypoxanthine	.000180
2-Deoxy-D-Ribose	.000300
D-Ribose	.000300
Xanthine	.000208

CuSO4 5H20	1.60E-08
ZnSO4 7H20	8.63E-04
Selenite Na	1.73E-05
Ferric Citrate	1.16E-03
MnSO4 H20	1.70E-08
NaSIO3 9H2O	1.40E-05
Molybdic Acid, Ammonium Salt	1.24E-07
NH4VO3	6.50E-08
NiSO4 6H2O	1.30E-08
SnCl2 (anhydrous)	1.20E-08
AlCl3 6H20	1.20E-07
AgNO3	1.70E-08
Ba(C2H3O2)2	2.55E-07
KBr	1.20E-08
CdCl2	2.28E-07
CoCl2	2.38E-07
CrCl3 (anhydrous)	3.20E-08
NaF	4.20E-07
GeO2	5.30E-08
KI	1.70E-08
RbCl	1.21E-07
ZrOCl2 8H2O	3.22E-07

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Claims

1. The defined serumfree medical solution consisting essentially of effective amounts of:

   a. an aqueous nutrient and electrolyte solution;
   b. a glycosaminoglycan;
   c. a deturgescent agent;
   d. an energy source;
   e. a buffer system;
   f. an antioxidant;
   g. membrane stabilizing agents;
   h. an antibiotic or antimycotic agent;
   i. ATP or energy precursors;
   j. nutrient cell supplements;
   k. coenzymes and enzyme supplements;
   l. nucleotide precursors;
   m. hormonal supplements;
   n. non-essential amino acids;
   o. trace minerals and trace elements and
   p. growth factors (animal, animal recombinant, human recombinant or natural).

2. The defined serumfree medical solution containing components which maintain and enhance the preservation of eye tissues at low to physiological temperatures (2°C to 38°C) with a physiological pH consisting essentially of effective amounts of:

   a. an aqueous nutrient and electrolyte solution;
   b. a glycosaminoglycan;
   c. a deturgescent agent;
   d. an energy source;
   e. a buffer system;
   f. an antioxidant;
   g. membrane stabilizing agents;
   h. an antibiotic or antimycotic agent;
   i. ATP or energy precursors;
   j. nutrient cell supplements;
   k. coenzymes and enzyme supplements;
   l. nucleotide precursors;
   m. hormonal supplements;
   n. non-essential amino acids;
   o. trace minerals and trace elements and
   p. growth factors (animal, animal recombinant, human recombinant or natural).

3. The defined serumfree medical solution containing components which maintain and enhance the preservation of eye tissues at low to physiological temperatures (16°C to 38°C) with a physiological pH consisting essentially of effective amounts of:

   a. an aqueous nutrient and electrolyte solution;
   b. a glycosaminoglycan;
   c. a deturgescent agent;
   d. an energy source;
   e. a buffer system;
   f. an antioxidant;
   g. membrane stabilizing agents;
   h. an antibiotic or antimycotic agent;
   i. ATP or energy precursors;
   j. nutrient cell supplements;
   k. coenzymes and enzyme supplements;
   i. nucleotide precursors;

m. hormonal supplements;
n. non-essential amino acids;
o. trace minerals and trace elements and
p. growth factors (animal, animal recombinant, human recombinant or natural).

4. The defined serumfree medical solution consisting essentially of effective amounts of:

a. An aqueous nutrient and electrolyte solution selected from the group of:

1. Minimal Essential Medium (MEM);
2. TC199 medium and
3. A combination of Minimal Essential Medium (MEM) and TC199 medium;

b. A glycosaminoglycan in the range of .001 mg/ml to 1.0 gram/ml selected from the group of:

1. chondroitin sulfate;
2. dermatin sulfate;
3. heparin sulfate;
4. heparan sulfate;
5 keratan sulfate and
6. hyaluronic acid;

c. A deturgescent agent in the range of .001 mg/ml to 1.0 gram/ml selected from the group of:

1. dextran;
2. dextran sulfate;
3. hydroxypropylmethyl cellulose;
4. carboxymethylcellulose;
5. cell gum;
6. sodium alginate;
7. albumin;
8. hydroxyethyl starch;
9. hydroxethyl cellulose;
10. dextrose;
11. glucose and
12. cyclodextrin;

d. An energy source in a range of .01 mM to 10 mM selected from the group of:

1. glucose;
2. pyruvate;
3. sucrose;
4. fructose and
5. dextrose;

e. A buffer system in a range of .01 mM to 10 mM selected from the group of:

1. sodium bicarbonate;
2. sodium acetate;
3. sodium citrate;
4. sodium phosphate and
5. HEPES buffer;

f. An antioxidant in a range of .001 μM to 10 mM selected from the group of:

1. L-ascorbic acid;
2. 2-mercaptoethanol;
3. glutathione;

4. alpha-tocopherol;
5. alpha-tocopherol acetate;
6. alpha-tocopherol phosphate and
7. selenium;

g. A membrane stabilizing component in a range of .001 pg/ml to 500 mg/ml selected from the group of:

1. vitamin A;
2. vitamin B;
3. retinoic acid;
4. trans-retinoic acid;
5. retinol acetate;
6. ethanolamine;
7. phosphoethanolamine;
8. transferrin;
9. lecithin;
10. B-sitosterol and
11. L-α-phosphatidyl choline;

h. An antibiotic an/or antimycotic in the range of .001 µg/ml to 100 mg/ml selected from the group of:

1. gentamycin;
2. kanamycin;
3. neomycin;
4. vancomycin;
5. obramycin;
6. clindamycin;
7. streptomycin;
8. levofloxacin;
9. penicillin;
10. cyclosporin;
11. amphotericin B and
12. nystatin;

i. ATP or energy precursors in the range of .001 mM to 10 mM selected from the group of:

1. adenosine;
2. inosine;
3. adenine;
4. flavin adenine dinucleotide;
5. uridine 5'-triphosphate Na;
6. 5' methylcytosine;
7. *B*-NAD and
8. *B*-NADP Na;

j. Nutrient cell supplements in a range of .001 µM to 10 mM selected from the group of:

1. alynyl-glutamine;
2. glycyl-glutamine;
3. L-amino-n-butyric acid;
4. L-arginine;
5. D-biotin;
6. Betaine HCl;
7. D-carnitine;
8. calciferol;
9. carotene;
10. cholesterol;
11. L-cystine;

12. L-cystiene;
13. L-glutamic acid;
14. D-glucosamine;
15. glucuronate;
16. D-Glucuronolactone;
17. L-hydroxyproline;
18. hypoxanthine;
19. L-inositol;
20. Glycine;
21. L-ornithine;
22. L-proline;
23. L-serine;
24. myo-inositol;
25. Menadione;
26. iacin;
27. nicotinic Acid;
28. p-amino benzoic acid;
29. D-Panthothenic Acid;
30. pyridoxal-5-phosphate;
31. pyridoxine HCl;
32. taurine;
33. thymidine;
34. xanthine and
35. Vitamin B12;

k. Coenzymes and enzyme supplements in a range of .001 μM to 10 mM selected from the group of:

1. acetyl Coenzyme A;
2. cocarboxylase;
3. coenzyme A;
4. coenzyme Q10 and
5. coenzyme K;

l. Nucleotide precursors in a range of .001 μM to 10 mM selected from the group of:

1. 2' Deoxyadenosine;
2. 2' Deoxycytidine HCL;
3. 2' Deoxyguanosine;
4. 2-deoxy-D-ribose and
5. D-ribose;

m. Hormonal supplements in a range of .001 pg/ml to 100 mg/ml selected from the group of:

1. *B*-estradiol;
2. progesterone;
3. testosterone;
4. cortisol;
5. Corticosterone;
6. thyroxine;
7. thyroid stimulating hormone and
8. calcitonin;

n. non-essential amino acids in the range of .001 μg/ml to 100 mg/ml selected from the group of:

1. L-alanine;
2. L-asparagine;
3. L-aspartic acid;
4. L-glutamic acid;

5. glycine;
6. L- proline and
7. L-serine;

o. Trace minerals and trace elements in the range of .001 pg/ml to .100 mg/ml selected from the group of:

1. $CuSO_4$ $5H_20$;
2. $ZnSO_4$ $7H_2O$;
3. Selenite Na;
4. Ferric citrate;
5. $MnSO_4$ H_20;
6. $NaSIO_3$ $9H_20$;
7. molybdic acid;
8. NH_4VO_3;
9. $NiSO_4$ $6H_2O$;
10. $SnCl_2$;
11. $AgNO_3$;
12. $Ba(C_2H_3O2)_2$;
13. KBr;
14. $CdCl_2$;
15. $CoCl_2$;
16. $CrCl_3$;
17. NaF;
18. GeO_2;
19. KL;
20. RbCl and
21. $ZrOCl_28H_20$;

p. Growth factors (animal, animal recombinant, human recombinant or natural) in the range of .001 pg/ml to .100 mg/ml selected from the group of:

1. PDGF-BB;
2. PDGF-AA;
3. Nerve growth factor;
4. Nerve growth factor B;
5. Stem cell factor;
6. Transforming growth factor-α;
7. Transforming growth factor-B;
8. Vascular endothelial growth factor;
9. B-endothelial cell growth factor;
10. Epidermal growth factor;
11. Epithelial neutrophil activating peptide;
12. Heparin binding EGF-like growth factor;
13. Fibroblastic growth factor-acidic;
14. Fibroblastic growth factor-basic;
15. IGF-I;
16. IGF-II;
17. Keratinocyte growth factor;
18. Platelet-derived endothelial cell growth factor
19. Insulin and
20. Hepatocyte growth factor

5. The defined serumfree medical solution containing components which maintain and enhance the preservation of eye tissues at low to physiological temperatures (2°C to 38°C) with a physiological pH consisting essentially of effective amounts of:

a. An aqueous nutrient and electrolyte solution selected from the group of:

1. Minimal Essential Medium (MEM);
2. TC199 medium and
3. A combination of Minimal Essential Medium (MEM) and TC199 medium;

b. A glyocsaminoglycan in the range of .001 mg/ml to 1.0 gram/ml selected from the group of:

1. chondroitin sulfate;
2. dermatin sulfate;
3. heparin sulfate;
4. heparan sulfate;
5 keratan sulfate and
6. hyaluronic acid;

c. A deturgescent agent in the range of .001 mg/ml to 1.0 gram/ml selected from the group of:

1. dextran;
2. dextran sulfate;
3. hydroxypropylmethyl cellulose;
4. carboxymethylcellulose;
5. cell gum;
6. sodium alginate;
7. albumin;
8. hydroxyethyl starch;
9. hydroxethyl cellose;
10. dextrose;
11. glucose and
12. cyclodextrin;

d. An energy source in a range of .01 mM to 10 mM selected from the group of:

1. glucose;
2. pyruvate;
3. sucrose;
4. fructose and
5. dextrose;

e. A buffer system in a range of .01 mM to 10 mM selected from the group of:

1. sodium bicarbonate;
2. sodium acetate;
3. sodium citrate;
4. sodium phosphate and
5. HEPES buffer;

f. An antioxidant in a range of .001 µM to 10 mM selected from the group of:

1. L-ascorbic acid;
2. 2-mercaptoethanol;
3. glutathione;
4. alpha-tocopherol;
5. alpha-tocopherol acetate;
6. alpha-tocopherol phosphate and
7. selenium;

g. A membrane stabilizing component in a range of .001 pg/ml to 500 mg/ml selected from the group of:

1. vitamin A;
2. vitamin B;

3. retinoic acid;
4. trans-retinoic acid;
5. retinol acetate;
6. ethanolamine;
7. phosphoethanolamine;
8. transferrin;
9. lecithin;
10. B-sitosterol and
11. L-α-phosphatidyl choline;

h. An antibiotic an/or antimycotic in the range of .001 µg/ml to 100 mg/ml selected from the group of:

1. gentamycin;
2. kanamycin;
3. neomycin;
4. vancomycin;
5. tobramycin;
6. clindamycin;
7. streptomycin;
8. levofloxacin;
9. penicillin;
10. cyclosporin;
11. amphotericin B and
12. nystatin;

i. ATP or energy precursors in the range of .001 mM to 10 mM selected from the group of:

1. adenosine;
2. inosine;
3. adenine;
4. flavin adenine dinucleotide;
5. uridine 5'-triphosphate Na;
6. 5' methylcytosine;
7. *B*-NAD and
8. *B*-NADP Na;

j. Nutrient cell supplements in a range of .001 µM to 10 mM selected from the group of:

1. alynyl-glutamine;
2. glycyl-glutamine;
3. L-amino-n-butyric acid;
4. L-arginine;
5. D-biotin;
6. Betaine HCl;
7. D-carnitine;
8. calciferol;
9. carotene;
10. cholesterol;
11 L-cystine;
12. L-cystiene;
13. L-glutamic acid;
14. D-glucosamine;
15. glucuronate;
16. D-Glucuronolactone;
17. L-hydroxyproline;
18. hypoxanthine;
19. L-inositol;
20. Glycine;

21. L-ornithine;
22. L-proline;
23. L-serine;
24. myo-inositol;
25. Menadione;
26. niacin;
27. nicotinic Acid;
28. p-amino benzoic acid;
29. D-Panthothenic Acid;
30. pyridoxal-5-phosphate;
31. pyridoxine HCl;
32. taurine;
33. Thymidine;
34. xanthine and
35. Vitamin B12;

k. Coenzymes and enzyme supplements in a range of .001 μM to 10 mM selected from the group of:

1. acetyl Coenzyme A;
2. cocarboxylase;
3. coenzyme A;
4. coenzyme Q10 and
5. coenzyme K;

l. Nucleotide precursors in a range of .001 μM to 10 mM selected from the group of;

1. 2' Deoxyadenosine;
2. 2' Deoxycytidine HCL;
3. 2' Deoxyguanosine;
4. 2-deoxy-D-ribose and
5. D-ribose;

m. Hormonal supplements in a range of .001 pg/ml to .100 mg/ml selected from the group of:

1. *B*-estradiol;
2. progesterone;
3. testosterone;
4. cortisol;
5. Corticosterone;
6. thyroxine;
7. thyroid stimulating hormone and
8. calcitonin;

n. non-essential amino acids in the range of .001 μg/ml to 100 mg/ml selected from the group of:

1. L-alanine;
2. L-asparagine;
3. L-aspartic acid;
4. L-glutamic acid;
5. glycine;
6. L- proline and
7. L-serine;

o. Trace minerals and trace elements in the range of .001 pg/ml to .100 mg/ml selected from the group of:

1. $CuSO_4$ $5H_20$;
2. $ZnSO_4$ $7H_2O$;
3. Selenite Na;

4. Ferric citrate;
5. $MnSO_4$ H_20;
6. $NaSlO_3$ $9H_20$;
7. molybdic acid;
8. NH_4VO_3;
9. $NiSO_4$ $6H_2O$;
10. $SnCl_2$;
11. $AgNO_3$;
12. $Ba(C_2H_3O2)_2$;
13. KBr;
14. $CdCl_2$;
15. $CoCl_2$;
16. $CrCl_3$;
17. NaF;
18. GeO_2;
19. KL;
20. RbCl and
21. $ZrOCl_28H_20$;

p. Growth factors (animal, animal recombinant, human recombinant or natural) in the range of .001 pg/ml to . 100 mg/ml selected from the group of:

1. PDGF-BB;
2. PDGF-AA;
3. Nerve growth factor;
4. Nerve growth factor B;
5. Stem cell factor;
6. Transforming growth factor-α;
7. Transforming growth factor-B;
8. Vascular endothelial growth factor;
9. B-endothelial cell growth factor;
10. Epidermal growth factor;
11. Epithelial neutrophil activating peptide;
12. Heparin binding EGF-like growth factor;
13. Fibroblastic growth factor-acidic;
14. Fibroblastic growth factor-basic;
15. IGF-I;
16. IGF-II;
17. Keratinocyte growth factor;
18. Platelet-derived endothelial cell growth factor
19. Insulin and
20. Hepatocyte growth factor

6. The defined serumfree medical solution containing components which maintain and enhance the preservation of eye tissues at low to physiological temperatures (16°C to 38°C) with a physiological pH consisting essentially of effective amounts of:

a. An aqueous nutrient and electrolyte solution selected from the group of:

1. Minimal Essential Medium (MEM);
2. TC199 medium and
3. A combination of Minimal Essential Medium (MEM) and TC199 medium;

b. A glycosaminoglycan in the range of .001 mg/ml to 1.0 gram/ml selected from the group of:

1. chondroitin sulfate;
2. dermatin sulfate;
3. heparin sulfate;

4. heparan sulfate;
5 keratan sulfate and
6. hyaluronic acid;

c. A deturgescent agent in the range of .001 mg/ml to 1.0 gram/ml selected from the group of:

1. dextran;
2. dextran sulfate;
3. hydroxypropylmethyl cellulose;
4. carboxymethylcellulose;
5. cell gum;
6. sodium alginate;
7. albumin;
8. hydroxyethyl starch;
9. hydroxethyl cellose;
10. dextrose;
11. glucose and
12. cyclodextrin;

d. An energy source in a range of .01 mM to 10 mM selected from the group of:

1. glucose;
2. pyruvate;
3. sucrose;
4. fructose and
5. dextrose;

e. A buffer system in a range of .01 mM to 10 mM selected from the group of:

1. sodium bicarbonate;
2. sodium acetate;
3. sodium citrate;
4. sodium phosphate and
5. HEPES buffer;

f. An antioxidant in a range of .001 µM to 10 mM selected from the group of:

1. L-ascorbic acid;
2. 2-mercaptoethanol;
3. glutathione;
4. alpha-tocopherol;
5. alpha-tocopherol acetate;
6. alpha-tocopherol phosphate and
7. selenium;

g. A membrane stabilizing component in a range of .001 pg/ml to 500 mg/ml selected from the group of:

1. vitamin A;
2. vitamin B;
3. retinoic acid;
4. trans-retinoic aoid;
5. retinol acetate;
6. ethanolamine;
7. phosphoethanolamine;
8. transferrin;
9. lecithin;
10. B-sitosterol and
11. L-α-phosphatidyl choline;

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h. An antibiotic an/or antlmycotic in the range of .001 μg/ml to 100 mg/ml selected from the group of:

1. gentamycin;
2. kanamycin;
3. neomycin;
4. vancomycin;
5. tobramycin;
6. clindamycin;
7. streptomycin;
8. levofloxacin;
9. penicillin;
10. cyclosporin;
11. amphotericin B and
12. nystatin;

i. ATP or energy precursors in the range of .001 mM to 10 mM selected from the group of:

1. adenosine;
2. inosine;
3. adenine;
4. flavin adenine dinucleotide;
5. uridine 5'-triphosphate Na;
6. 5' methylcytosine;
7. *B*-NAD and
8. *B*-NADP Na;

j. Nutrient cell supplements in a range of .001 μM to 10 mM selected from the group of:

1. alynyl-glutamine;
2. glycyl-glutamine;
3. L-amino-n-butyric acid;
4. L-arginine;
5. D-biotin;
6. Betaine HCl;
7. D-carnitine;
8. calciferol;
9. carotene;
10. cholesterol;
11 L-cystine;
12. L-cystiene;
13. L-glutamic acid;
14. D-glucosamine;
15. glucuronate;
16. D-Glucuronolactone;
17. L-hydroxyproline;
18. hypoxanthine;
19. L-inositol;
20. Glycine;
21. L-ornithine;
22. L-proline;
23. L-serine;
24. myo-inositol;
25. Menadione;
26. niacin;
27. nicotinic Acid;
28. p-amino benzoic acid;
29. D-Panthothenic Acid;
30. pyridoxal-5-phosphate;

31. pyridoxine HCl;
32. taurine;
33. thymidine;
34. xanthine and
35. Vitamin B12;

k. Coenzymes and enzyme supplements in a range of .001 μM to 10 mM selected from the group of:

1. acetyl Coenzyme A;
2. cocarboxylase;
3. coenzyme A;
4. coenzyme Q10 and
5. coenzyme K;

l. Nucleotide precursors in a range of .001 μM to 10 mM selected from the group of:

1. 2' Deoxyadenosine;
2. 2' Deoxycytidine HCL;
3. 2' Deoxyguanosine;
4. 2-deoxy-D-ribose and
5. D-ribose;

m. Hormonal supplements in a range of .001 pg/ml to .100 mg/ml selected from the group of:

1. *B*-estradiol;
2. progesterone;
3. testosterone;
4. cortisol;
5. Corticosterone;
6. thyroxine;
7. thyroid stimulating hormone and
8. calcitonin;

n. non-essential amino acids in the range of .001 μg/ml to 100 mg/ml selected from the group of:

1. L-alanine;
2. L-asparagine;
3. L-aspartic acid;
4. L-glutamic acid;
5. glycine;
6. L- proline and
7. L-serine;

o. Trace minerals and trace elements in the range of .001 pg/ml to .100 mg/ml selected from the group of:

1. $CuSO_4$ $5H_20$;
2. $ZnSO_4$ $7H_2O$;
3. Selenite Na;
4. Ferric citrate;
5. $MnSO_4$ H_20;
6. $NaSiO_3$ $9H_20$;
7. molybdic acid;
8. NH_4VO_3;
9. $NiSO_4$ $6H_2O$;
10. $SnCl_2$;
11. $AgNO_3$;
12. $Ba(C_2H_3O2)_2$;
13. KBr;

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14. $CdCl_2$;
15. $CoCl_2$;
16. $CrCl_3$;
17. NaF;
18. GeO_2;
19. KL;
20. RbCl and
21. $ZrOCl_2 8H_20$;

p. Growth factors (animal, animal recombinant, human recombinant or natural) in the range of .001 pg/ml to . 100 mg/ml selected from the group of:

1. PDGF-BB;
2. PDGF-AA;
3. Nerve growth factor;
4. Nerve growth factor B;
5. Stem cell factor;
6. Transforming growth factor-α;
7. Transforming growth factor-B;
8. Vascular endothelial growth factor;
9. B-endothelial cell growth factor;
10. Epidermal growth factor;
11. Epithelial neutrophil activating peptide;
12. Heparin binding EGF-like growth factor;
13. Fibroblastic growth factor-acidic;
14. Fibroblastic growth factor-basic;
15. IGF-I;
16. IGF-II;
17. Keratinocyte growth factor;
18. Platelet-derived endothelial cell growth factor
19. Insulin and
20. Hepatocyte growth factor.

7. The defined serumfree medical solution consisting essentially of effective amounts of:

a. an aqueous nutrient and electrolyte solution;
b. a glycosaminoglycan;
c. a deturgescent agent;
d. an energy source;
e. a buffer system;
f. an antioxidant;
g. membrane stabilizing agents;
h. an antibiotic or antimycotic agent;
i. ATP or energy precursors;
j. nutrient cell supplements;
k. coenzymes and enzyme supplements;
l. nucleotide precursors;
m. hormonal supplements;
n. non-essential amino acids and
o. trace minerals and trace elements.

8. The defined serumfree medical solution consisting essentially of effective amounts of:

a. An aqueous nutrient and electrolyte solution selected from the group of:

1. Minimal Essential Medium (MEM);
2. TC199 medium and
3. A combination of Minimal Essential Medium (MEM) and TC199 medium;

b. A glycosaminoglycan in the range of .001 mg/ml to 1.0 gram/ml selected from the group of:

1. chondroitin sulfate;
2. dermatin sulfate;
3. heparin sulfate;
4. heparan sulfate;
5 keratan sulfate and
6. hyaluronic acid;

c. A deturgescent agent in the range of .001 mg/ml to 1.0 gram/ml selected from the group of:

1. dextran;
2. dextran sulfate;
3. hydroxypropylmethyl cellulose;
4. carboxymethylcellulose;
5. cell gum;
6. sodium alginate;
7. albumin;
8. hydroxyethyl starch;
9. hydroxethyl cellose;
10. dextrose;
11. glucose and
12. cyclodextrin;

d. An energy source in a range of .01 mM to 10 mM selected from the group of:

1. glucose;
2. pyruvate;
3. sucrose;
4. fructose and
5. dextrose;

e. A buffer system in a range of .01 mM to 10 mM selected from the group of:

1. sodium bicarbonate;
2. sodium acetate;
3. sodium citrate;
4. sodium phosphate and
5. HEPES buffer;

f. An antioxidant in a range of .001 μM to 10 mM selected from the group of:

1. L-ascorbic acid;
2. 2-mercaptoethanol;
3. glutathione;
4. alpha-tocopherol;
5. alpha-tocopherol acetate;
6. alpha-tocopherol phosphate and
7. selenium;

g. A membrane stabilizing component in a range of .001 pg/ml to 500 mg/ml selected from the group of:

1. vitamin A;
2. vitamin B;
3. retinoic acid;
4. trans-retinoic acid;
5. retinol acetate;
6. ethanolamine;

7. phosphoethanolamine;
8. transferrin;
9. lecithin;
10. B-sitosterol and
11. L-α-phosphatidyl choline;

h. An antibiotic an/or antimycotic in the range of .001 µg/ml to 100 mg/ml selected from the group of:

1. gentamycin;
2. kanamycin;
3. neomycin;
4. vancomycin;
5. tobramycin;
6. clindamycin;
7. streptomycin;
8. levofloxacin;
9. penicillin;
10. cyclosporin;
11. amphotericin B and
12. nystatin;

i. ATP or energy precursors in the range of .001 mM to 10 mM selected from the group of:

1. adenosine;
2. inosine;
3. adenine;
4. flavin adenine dinucleotide;
5. uridine 5'-triphosphate Na;
6. 5' methylcytosine;
7. *B*-NAD and
8. *B*-NADP Na;

j. Nutrient cell supplements in a range of .001 µM to 10 mM selected from the group of:

1. alynyl-glutamine;
2. glycyl-glutamine;
3. L-amino-n-butyric acid;
4. L-arginine;
5. D-biotin;
6. Betaine HCl;
7. D-carnitine;
8. calciferol;
9. carotene;
10. cholesterol;
11 L-cystine;
12. L-cystiene;
13. L-glutamic acid;
14. D-glucosamine;
15. glucuronate;
16. D-Glucuronolactone;
17. L-hydroxyproline;
18. hypoxanthine;
19. L-inositol;
20. Glycine;
21. L-ornithine;
22. L-proline;
23. L-serine;
24. myo-inositol;

25. Menadione;
26. niacin;
27. nicotinic Acid;
28. p-amino benzoic acid;
29. D-Panthothenic Acid;
30. pyridoxal-5-phosphate;
31. pyridoxine HCl;
32. taurine;
33. thymidine;
34. xanthine and
35. Vitamin B12;

k. Coenzymes and enzyme supplements in a range of .001 μM to 10 mM selected from the group of:

1. acetyl Coenzyme A;
2. cocarboxylase;
3. coenzyme A;
4. coenzyme Q10 and
5. coenzyme K;

l. Nucleotide precursors in a range of .001 μM to 10 mM selected from the group of:

1. 2' Deoxyadenosine;
2. 2' Deoxycytidine HCL;
3. 2' Deoxyguanosine;
4. 2-deoxy-D-ribose and
5. D-ribose;

m. Hormonal supplements in a range of .001 pg/ml to .100 mg/ml selected from the group of:

1. *B*-estradiol;
2. progesterone;
3. testosterone;
4. cortisol;
5. Corticosterone;
6. thyroxine;
7. thyroid stimulating hormone and
8. calcitonin;

n. non-essential amino acids in the range of .001 μg/ml to 100 mg/ml seleoled from the group of:

1. L-alanine;
2. L-asparagine;
3. L-aspartic acid;
4. L-glutamic acid;
5. glycine;
6. L- proline and
7. L-serine;

o. Trace minerals and trace elements in the range of .001 pg/ml to .100 mg/ml selected from the group of:

1. $CuSO_4$ $5H_20$;
2. $ZnSO_4$ $7H_2O$;
3. Selenite Na;
4. Ferric citrate;
5. $MnSO_4$ H_2O;
6. $NaSIO_3$ $9H_20$
7. molybdic acid;

8. NH_4VO_3;
9. $NISO_4\ 6H_2O$;
10. $SnCl_2$;
11. $AgNO_3$;
12. $Ba(C_2H_3O2)_2$;
13. KBr;
14. $CdCl_2$;
15. $CoCl_2$;
16. $CrCl_3$;
17. NaF;
18. GeO_2;
19. KL;
20. RbCl and
21. $ZrOC_{12}8H_2O$.

9. The defined serumfree medical solution consisting essentially of one or more effective amounts of:

a. an aqueous nutrient and electrolyte solution;
b. a glycosaminoglycan;
c. a deturgescent agent;
d. an energy source;
e. a buffer system;
f. an antioxidant;
g. membrane stabilizing agents;
h. an antibiotic or antimycotic agent;
i. ATP or energy precursors;
j. nutrient cell supplements;
k. coenzymes and enzyme supplements;
l. nucleotide precursors;
m. hormonal supplements;
n. non-essential amino acids;
o. trace minerals and trace elements and
p. growth factors (animal, animal recombinant, human recombinant or natural).

10. A pharmaceutical composition of claim 9 wherein said solution maintains and enhances the preservation of mammalian eye tissues after coming into contact with said solution.

11. A pharmaceutical composition of claim 9 wherein said solution maintains and enhances the preservation of mammalian eye-tissues after coming into contact with said solution before or after surgical use of a laser.

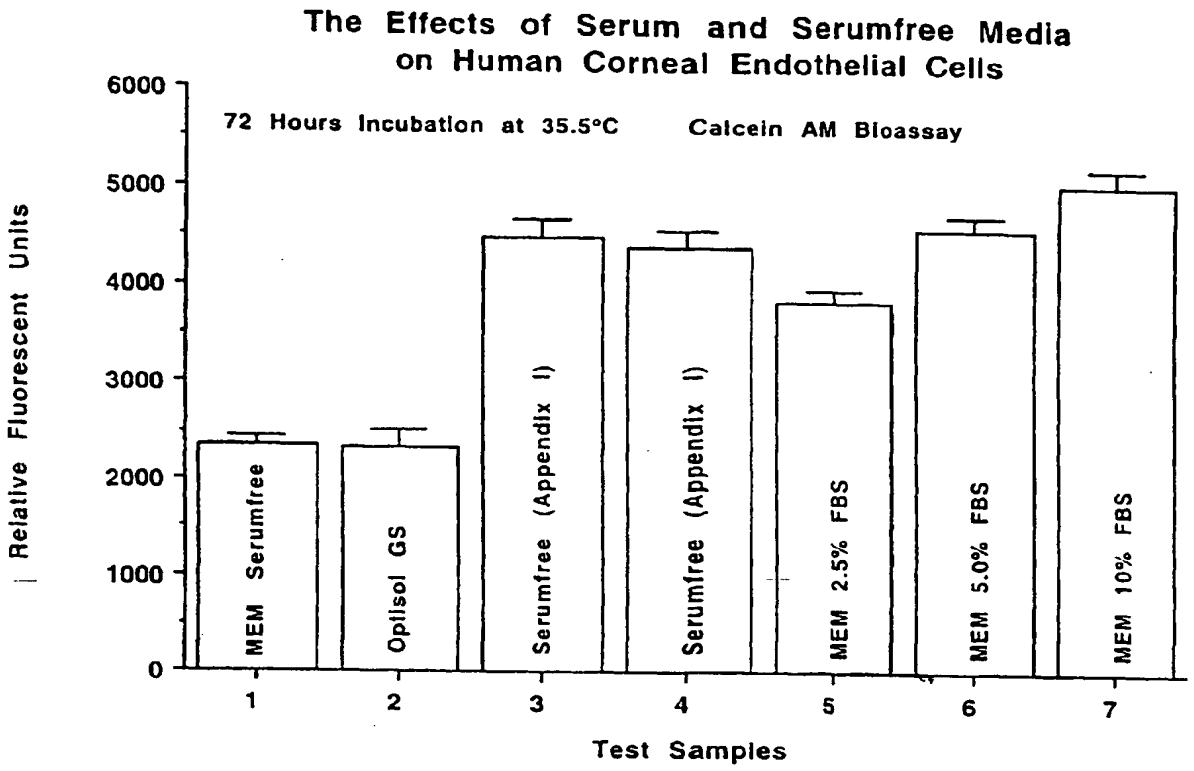
12. A pharmaceutical composition of claim 9 wherein said solution maintains and enhances the preservation of mammalian eye tissues after coming into contact with said solution before or after degenerative eye conditions.

13. A pharmaceutical composition of claim 9 wherein said solution maintains and enhances the preservation of mammalian eye tissues after coming into contact with said solution before or after surgery.

14. A pharmaceutical composition of claim 9 wherein said solution maintains and enhances the preservation of mammalian tissues after coming into contact with said solution.

15. A method of treating an eye tissue for use in eye surgery, characterised in that it comprises keeping the tissue in contact with a solution as claimed in any of the preceding claims in the period elapsing between removing the tissue from a donor and implanting in into a recipient.

FIGURE 1



NSDOCID: <EP 1000541A1 I >

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EUROPEAN SEARCH REPORT

Application Number
EP 99 30 8702

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A01N
C12N

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
THE HAGUE	9 March 2000	Decorte, D

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